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TITLE: Allele Imbalance or Loss of Heterozygosity in Normal-Appearing Breast Epithelium as a Novel Marker to Predict Future Breast Cancer

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14. ABSTRACT

The goal of this study is to determine whether the occurrence of AI/LOH in the DNA of histologically normal epithelium from non-cancerous breasts predicts future breast cancer development. If so, then AI/LOH would be an excellent candidate molecular marker of increased sporadic breast cancer risk: its incidence increases during cancer development, it can be quantified and standardized, it is likely to reflect dysregulation of genetic mechanisms that could be potential targets for pharmacological modulation. In the past year, we have made substantial progress on the first two of our four aims. From the Nurses' Health Study Benign Breast Disease nested case-control substudy, cases and matched controls have been identified and their tissue blocks sent to the PI's laboratory for study; and the techniques to quantitate AI/LOH in an automated fashion are being finalized. In the next year, we anticipate continued progress on the tasks as outlined in the statement of work.

15. SUBJECT TERMS
allele imbalance, loss of heterozygosity, normal epithelium, breast cancer, predictive marker

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Introduction.
Over the past year, we have made considerable progress in achieving the aims of the funded project. Our progress is described below, by each task in the statement of work. In addition, one leadership change should be noted. Since the study was funded, one of our Nurses' Health Study - Benign Breast Disease substudy (NHS-BBD) collaborators, Dr Graham Colditz, has switched institutions. However, this has not affected the study, since his role has been taken over by one of the NHS-BBD co-investigators, Dr. Rulla Tamimi. Dr Tamimi had been involved in planning this project prior to Dr. Colitz's departure and she has continued active participation, as evidenced by her work on Task 1.

Body.
Task 1. Identify 70 subjects from the Nurses' Health Study Benign Breast Disease (NHS-BBD) nested case-control study who had benign breast biopsies with available tissue blocks and full clinical follow-up. Months 1-16.

This task is nearly complete. Dr Tamimi selected and assembled the benign breast disease tissue blocks. As planned, she selected breast cancer cases from the nested case-control study of benign breast disease and breast cancer who were between ages 35 and 55 at the time of the benign biopsy, had no atypia on the benign biopsy, and had no family history of breast cancer. For each breast cancer case, she selected one control who was matched as closely as possible to the case on age at benign biopsy, year of biopsy and who also lacked atypia on the biopsy and a family history of breast cancer. A total of 44 cases and 44 controls (with a total of 343 tissue blocks) met the criteria. Extra cases and controls are included because when the blocks are examined in the lab, some will be found to be unusable for technical reasons such as: no or too few normal lobules, or degraded DNA. The 343 blocks were sent to the Rosenberg lab blinded as to identity and outcome. The blocks are stored in a locked room accessible only to the PI and one laboratory associate. We are in the process are developing spreadsheets to track the blocks, and examining each block to make sure that it is physically able to cut, and confirming that the sections cut from the block contain normal epithelium.

Task 2: Microdissect multiple normal-appearing epithelial samples (TDLU) from each eligible NHS-BBD subject's blocks, extract DNA, quantify AI using an optimized marker panel and a high-throughput system. Months 1-33

2A: Optimize panel of ~ 25 markers to be used. Months 1-12. We have made progress on this aim. Specifically, we have switched from a radioactive, gel and autoradiography-based detection system to a fluorescent, capillary electrophoresis-based detection system. This detection system is more automated, and therefore permits higher through-put for detecting AI/LOH. To test this newer system, we examined a series of control and tumor samples for AI/LOH using both approaches. As seen in the example shown in **Figure 1** (next page), the two approaches produced equivalent AI/LOH ratios. We will be proceeding using the fluorescent, capillary-based system, which is faster, safer and most commonly used in similar research.

In addition, we have just begun the process of optimizing our marker panel. Our plan remains to purchase fluorescently labeled primers from those already available at ABI. We were originally going to custom order markers that were not commercially available, but it appears that most markers we are considering are commercially available. We anticipate that we will soon begin to test primers using template DNA from microdissected, FFPE normal breast epithelium from tissues not associated with the NHS-BBD substudy.

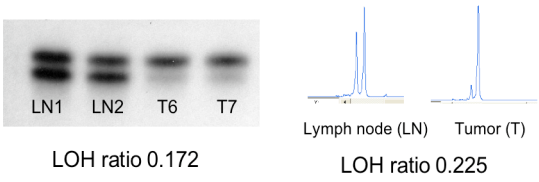

Figure 1. Equivalent results from a radioactive-label, gel-based detection system (left panel) and a fluorescent-label, capillary-electrophoresis based detection system

2B: Section NHS-BBD blocks, microdissect normal epithelium, isolate DNA, perform PCRs, run reaction products through capillary electrophoresis. Months 6- 33. We have begun this part of the aim. Specifically, we have begun to section the first batch of NHS-BBD blocks and we are determining which of these blocks contain adequate lesions to begin microdissection and DNA isolation.

2C: Enter results onto Excel spreadsheet, send spreadsheet to statistician. Months 12-33. We have not yet begun this section of the task.

Task 3. Correlate prevalence and chromosome sites of AI with clinical outcome. Months 12 – 36. We have not yet begun this task.

Task 4. Write-up and presentation of results. Months 24-36. We have not yet begun this task.

Key Research Accomplishments

> NHS BBD substudy cases and controls identified and blocks pulled; blocks sent blinded as to identify and outcome to the Rosenberg lab; blocks received and stored as required by regulations.

> switch made from radioactive gel-based system to fluorescent capillary electrophoresis based system to detect AI/LOH.

Reportable Outcomes

None

Conclusions

The goal of this study is to determine whether the occurrence of AI/LOH in histologically normal epithelium from non-cancerous breasts predicts future breast cancer development. In the past year, we have made substantial progress on the first two of our aims. The cases and controls have been identified and their tissue blocks sent to the laboratory where they will be studied; and the techniques to study the AI/LOH in a more automated fashion are being finalized. We anticipate successful completion of the project within the planned timeline.

References

None.

Appendices

None